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(54) Title: COMPOSITIONS OF LIPOSOMES AND β_2-RECEPTOR ACTIVE SUBSTANCES

(57) Abstract

A pharmaceutical composition consisting of a dry powder comprising liposomes and β_2-receptor active substance and processes for preparation of such a composition. The unique anti-allergic, broncho-dilating and anti-inflammatory activities in the respiratory tract by the use of the said compositions are further described.

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Compositions of liposomes and β_2-receptor active substances

DESCRIPTION

Field of the invention

The present invention relates to a novel pharmaceutical composition in dry powder form and is particularly concerned with liposomal formulations of β_2-receptor active substances for inhalation.

The object of the invention is to provide a pharmaceutical composition consisting of a dry powder comprising a β_2-receptor active substance encapsulated into liposomes. By encapsulating a β_2-receptor active substance into liposomes, it is possible to prolong the retention of this group of substances in the lung and hence to increase the duration and efficacy of the anti-inflammatory, broncho-dilating and anti-allergic activities.

One of the major problems in the development of a pharmaceutical liposomal formulation is the long-time stability. Aqueous liposome dispersions have a limited physical stability since the liposomes can aggregate resulting in a change in the size distribution. Furthermore, if the encapsulated drug is hydrophilic it may be lost into the external aqueous phase. In addition, there is a potential risk for chemical degradation of the lipid components and the pharmacologically active substance in an aqueous milieu. The problem concerning stability can to large extent be solved if a dry solid composition is developed.

The following β_2-receptor active substances are examples of substances which can be used in accordance with the present invention: terbutaline, salbutamol, mabuterol, fenoterol,

orciprenaline, formoterol, isoprenaline, isoetharine, clenbuterol, hexoprenaline, procaterol, 1-(4-hydroxyphenyl--2-[1,1-dimethyl-3-(2-methoxy-phenyl)propylamino]-ethanol, 1-(3,5-dihydroxy-phenyl)-2-[1,1-dimethyl-3-(2-methoxyphenyl)-propylamino]-ethanol, 1-(3,4-dihydroxyphenyl)-2-[1,1--dimethyl-3-(2-methoxyphenyl)propylamino]ethanol, (4-hydroxy-α'-[[[6-(4-phenylbutoxy)-hexyl]-amino]-methyl]--1,3-benzyl-dimethanol, pharmacologically acceptable salts thereof and compounds of similar pharmacological properties. Preferred pharmacologically acceptable salts of β_2-receptor substances are salts with physiologically acceptable acids. Suitable acids which may be used are, for example, hydrochloric, hydrobromic, sulfuric, fumaric, citric, tartaric, maleic or succinic acid. The β_2-receptor active substance which is particularly preferred is terbutaline sulphate.

Background art

Inhalation of β_2-receptor active substances is used for the treatment of allergic and inflammatory conditions in the respiratory tract, like asthma and airway hyperresponsiveness. However, the treatment suffers from the disadvantage that it has a limited duration of action. For example, the bronchodilating effect of inhaled terbutaline sulphate administered during the evening is lost during the late night which might result in a new asthmatic attack during the sleeping period.

Liposomes are widely described in the litterature and their general structure is well known; they are structures composed of concentric rings of lipid bilayers. Dehydrated liposomes are described in International Application WO86/01103 (Liposome Co.). Liposomes have been used as carriers for different kinds of pharmacologically active drugs in order to improve the therapeutic efficacy. Drug-loaded liposomal formulations are however generally intended for subcutanous, intravenous or oral administration. Drug encapsulated into liposomes intended specifically for inhalation are for instance described in European Patent Applications 158441 (Phares), 84898 (Fison) and 0170642 (Draco) and in International Application WO86/01714 (Riker).

Disclosure of the invention

The lipid materials used in the present invention may be any of those conventionally used in liposomal formulations. Usually the main liposome-forming component is a phosholipid, including synthetic lecithins and natural lecithins,

e.g. those derived from egg and soyabean. The phase-transition temperature (Tc) of the phospholipid can have a marked influence on the retention of the liposome encapsulated substance in the target organ. It is therefore favourable to use well-defined synthetic phospholipids. Dimyristoyl phosphatidylcholine, DMPC (Tc = 23 °C), dipalmitoyl phosphatidylcholine, DPPC (Tc = 41 °C) and distearoyl phosphatidylcholine, DSPC (Tc = 55 °C), either alone or in combination are preferred to the natural lecithins. It is known that DPPC is the main phospholipid in the natural lung-surfactant. By the use of pure synthetic phospholipids the risk of undesired immunological reactions is minimized.

In addition to the main liposome-forming component other lipids may be used to optimize the properties of the formulation. Examples of such additives are cholesterol and components which provide positive or negative charge.

Cholesterol, or carbohydrate derivatives thereof in a proportion up to 50 % w/w of the total lipids may be incorporated to modify the membrane structure rendering it more fluid or more rigid and thereby influence the release properties of the entrapped pharmacologically active material. Cholesterol also has a positive effect on the stability of the liposomes during lyophilization.

Components which provides a negative or positive charge may be incorporated in a proportion up to 30 % w/w of the total lipids. They will provide an electrostatic stabilization of the liposome dispersion and may also optimize the uptake of the liposomes in the target cells. Examples of negatively charged lipophilic substances are phosphatic acid, dicetyl phosphoric acid, phosphatidyl serine, phosphatidyl inositol, phosphatidyl glycerol and phosphatidyl ethanolamine. Examples of positively charged lipophilic substances are stearylamine, stearylamine acetate and cetylpyridinium chloride.

The initial stages of the preparation of liposomes according to the present invention may conveniently follow any procedure which results in the encapsulation of a hydrophilic substance into liposomes.

Examples of such methods are the

A reverse phase evaporation (US Patent 4,235,871)

B dehydration-rehydration method (Kirby, C and Gregoriadis, G; Bio/Technology, Nov 1984, 979-984)

C film method (Bangham et al J Mol Biol 1965, 13, 238-252)

D freeze-drying method (UK Patent 1,573,343)

Method A

The lipid materials are dissolved in an organic solvent and the β_2-receptor active substance is dissolved in an aqueous phase. The two solutions are mixed to produce an emulsion of the water-in-oil type. The organic solvent is removed and the resulting gel is suspended in an aqueous solution to give a liposome dispersion.

Method B

The lipid material is dissolved in a solvent e.g. chloroform or t-butanol, and are evaporated to a thin lipid film (chloroform) or freeze-dried (t-butanol). Distilled water is added and the temperature is raised. The final temperature will be above the phase-transition temperature of the lipid material. The resulting liposome dispersion is mixed with an aqueous solution of a β_2-receptor active substance. It is often appropriate to use 0.1 to 10 parts by weight of β_2-receptor active substance per part of lipid material.

The mixture is freeze-dried, and the dry material is dispersed in a minimal amount of distilled water. The temperature is raised above the phase-transition temperature of the lipid material. After equilibration, the dispersion is diluted with additional aqueous solution. The resulting liposomes will be in a range of sizes (50 nm - 10 μm).

Method C

The lipid materials are dissolved in a solvent, e.g. chloroform or ethanol and the solvent is evaporated. Liposomes are formed by adding an aqueous solution of a β_2-receptor active substance and raising the temperature above the phase-transition temperature of the lipid material. It is often appropriate to use 0.1 to 10 parts by weight of β_2-receptor active substance per part of lipid material. The concentration of β_2-receptor active substance during liposome formation should be 1 - 100 mg/ml. The resulting liposomes will be in a range of sizes (50 nm - 10 μm).

Method D

The lipids and the β_2-receptor active substance are dissolved in a solvent, e.g. a mixture of t-butanol and water, and freeze-dried. It is often appropriate to use 0.1 to 10 parts by weight of β_2-receptor active substance per part of lipid material. The resulting freeze-dried powder is dispersed in a minimal amount of distilled water and the temperature is raised. The final temperature will be above the phase-transition temperature of the lipid material. After equilibration, the dispersion is diluted with additional aqueous solution. The resulting liposomes will be in a range of sizes (50 nm to 10 μm).

Regardless the method used for formation of the liposomes there will be significant amounts of drug not encapsulated

into the liposomes but remaining in the continuous aqueous phase. It may be desirable to remove the drug (or a fraction of it) from the continuous phase and this is conveniently done either by dialysing the liposomal formulation against a drug-free aqueous phase, by centrifugation of the liposome dispersion of by chromatography using an ion-exchange resin capable of selectivly binding the non-entrapped β_2-receptor active substance.

Preparation of dry liposomal powder containing β_2-receptor active substance

Since aqueous dispersions of liposomes have a limited stability, it may be favourable to remove the solvent from preparations intended for long-time storage. The dehydration can be performed in a number of different ways, e.g. spray-drying and lyophilization. Lyophilization is particulary preferred. In that case the liposome dispersions described in the present invention are mixed with a cryoprotective agent such as a carbohydrate, e.g. lactose or threhalose at the concentration of 0 to 95 % by weight of the final composition and are rapidly frozen in liquid nitrogen. We find the rapid freezing step important to preserve the liposomal structure. After lyophilization a dry powder suitable for long-time storage is obtained. The liposome dispersion can be reconstituted after addition of an aqueous solution to the said powder.

Determination of the percentage β_2-receptor active substance associated with liposomes

The equilibrated liposome dispersion containing the β_2-receptor active substance is, if necessary, diluted with an appropriate aqueous solution (distilled water, saline etc) and centrifuged at 25000 g to 100000 g for 15 min to 1

hour. Aliquots of the supernatant and the liposomal pellet (suspended in distilled water) are dissolved in t-butanol and assayed in a Varian DMS 100 spectrophotometer at the following wavelengths; terbutaline sulphate 280 nm, salbutamol 276 nm, mabuterol 240 nm, 1-(4-hydroxyphenyl)--2-[1,1-dimethyl-3-(2-methoxyphenyl) propylamino]-ethanol, 270 nm and procaterol 257 nm. The percentage of β_2-receptor active substance encapsulated in the liposomes (the encapsulation efficacy) is calculated as follows:

$$\frac{[\beta_2\text{-recept act subst in pel}] \times 100}{[\beta_2\text{-recept act subst in pel}] + [\beta_2\text{-recept act subst in supern.}]}$$

Working examples

The present invention is exemplified but in no way limited by the following examples.

Compositions

Example 1

DPPC (10 mg), DPPA (Dipalmitoyl phosphatic acid) (1 mg) and cholesterol (10 mg) are mixed in a glass tube. All components are dissolved in chloroform. The solvent is evaporated by the use of N_2, which results in a thin film of the lipid components on the inner surface of the glass tube. 1 ml of an aqueous solution of terbutaline sulphate (10 mg/ml) is added to the lipids. Liposomes are formed by shaking the glass tube at 60 °C for 30 minutes. The encapsulation of terbutaline sulphate into the liposomes was 10 % according to the method described above.

Example 2

One gram of Epikuron 200H (Lucas Meyer, Hamburg) and one gram of terbutaline sulphate are dispersed, under gentle heating, in t-butanol (30 ml) and distilled water is added until the components are completely dissolved. The solution is frozen and lyophilized. 40 mg of the dry lyophilized powder is dispersed in 200 µl distilled water and the dispersion is heated (60 °C) for 30 minutes. Thereafter 2.8 ml distilled water is added. The percentage of terbutaline sulphate encapsulated into the liposomes was 51 % according to the method described above.

Example 3

In a 50 ml round bottom flask 60 mg of DPPC and 60 mg of cholesterol are dissolved in 10 g chloroform. 60 mg of terbutaline sulphate is dissolved in 1 g of distilled water. The terbutaline sulphate solution is added to the flask and the two solutions are emulsified with an Ultra-Turrax. The resulting emulsion is evaporated on a Buchi rotary evaporator until a gel is formed. To the gel 3 g of distilled water is added and the sample is mixed until a liposome dispersion forms. The encapsulation of terbutaline sulphate into the liposomes was 38 % according to the method described above.

Examples 4 - 14

The desired quantities of the appropriate lipids (see below) are mixed in a glass tube. All components are dissolved in a small quantity of chloroform and evaporated to dryness to leave a thin lipid film on the inner surface of the glass tube. Distilled water (4 ml) is added to the lipid film and liposomes are formed by sonificating the sample at elevated temperature. Terbutaline sulphate is

dissolved in 2 ml distilled water. The liposome dispersion and the drug solution are mixed, frozen and lyophilized. The dry product is dispersed in 100 µl distilled water per 10 mg phospholipid. Liposomes are formed by heating (60 °C) the sample for 30 minutes. The encapsulation of drug into the liposomes is determined according to the method described above.

The following liposome compositions were prepared using the above general procedure:

			Encapsulation efficacy (%)
4)	DPPC	10 mg	43
	Terbutaline sulphate	10 mg	
5)	DPPC	10 mg	
	Cholesterol	10 mg	40
	Terbutaline sulphate	10 mg	
6)	DPPC	10 mg	
	Stearylamine	1 mg	
	Cholesterol	10 mg	43
	Terbutaline sulphate	10 mg	
7)	DPPC	10 mg	
	Phosphatidyl serine	1 mg	
	Cholesterol	10 mg	36
	Terbutaline sulphate	10 mg	
8)	DPPC	9 mg	
	DPPA	1 mg	
	Cholesterol	10 mg	42
	Terbutaline sulphate	10 mg	

9)	DPPC	9 mg	
	DPPA	1 mg	
	Cholesterol	1 mg	31
	Terbutaline sulphate	10 mg	
10)	DPPC	9 mg	
	DPPA	1 mg	28
	Terbutaline sulphate	10 mg	
11)	DMPC	9 mg	
	DPPA	1 mg	47
	Cholesterol	10 mg	
	Terbutaline sulphate	10 mg	
12)	DSPC	9 mg	
	DPPA	1 mg	
	Cholesterol	10 mg	64
	Terbutaline sulphate	10 mg	
13)	Egg-lecithin	40 mg	
	DPPA	5 mg	37
	Cholesterol	50 mg	
	Terbutaline sulphate	50 mg	
14)	Egg-lecithin	9 mg	
	DPPA	1 mg	40
	Cholesterol	10 mg	
	Terbutaline sulphate	10 mg	

Examples 15 - 18

Liposomes with various β_2-receptor active substances are prepared according to the method described in examples 4 - 14 and the fraction of the drug encapsulated into the liposomes is determined according to the method described above.

			Encapsulation efficacy (%)
15)	DPPC	40 mg	
	DPPA	4 mg	38
	Cholesterol	40 mg	
	Mabuterol	40 mg	
16)	DPPC	40 mg	
	DPPA	4 mg	45
	Cholesterol	40 mg	
	Salbutamol	40 mg	
17)	DPPC	40 mg	
	DPPA	4 mg	
	Cholesterol	40 mg	20
	1-(4-hydroxyphenyl)-2-[1,1-dimethyl-3-(2-methoxy-phenyl) propylamino]-ethanol	40 mg	
18)	DPPC	40 mg	
	DPPA	4 mg	
	Cholesterol	40 mg	35
	Procaterol	40 mg	

Examples 19 - 22

Liposome dispersions are prepared according to the method described in examples 4 - 14. The amount of lipid material was kept constant while the amount of β_2-receptor active substance (in this case terbutaline sulphate) was varied.

The following liposome compositions were prepared:

			Encapsulation efficacy (%)	Absolute amount of terbutaline sulphate encapsulated into liposomes (mg)
19)	DPPC	9 mg		
	DPPA	1 mg	57	2.9
	Cholesterol	10 mg		
	Terbutaline sulphate	5 mg		
20)	DPPC	9 mg		
	DPPA	1 mg	39	3.9
	Cholesterol	10 mg		
	Terbutaline sulphate	10 mg		
21)	DPPC	9 mg		
	DPPA	1 mg	27	5.4
	Cholesterol	10 mg		
	Terbutaline sulphate	20 mg		
22)	DPPC	9 mg		
	DPPA	1 mg	19	5.7
	Cholesterol	10 mg		
	Terbutaline sulphate	30 mg		

Dry powder

Example 23

Liposomes are prepared according to examples 4 - 14. The liposome dispersion (100 µl) is diluted to 1.5 ml with an aqueous solution of lactose (100 mg/ml). The dispersion is flash-frozen by dripping it into liquid nitrogen and is then lyophilized. The dry powder is dispersed in distilled water and the encapsulation of terbutaline sulphate into the liposomes is calculated according to the method described above.

		Encapsulation efficacy (%)
DPPC	10 mg	
Cholesterol	10 mg	20
Terbutaline sulphate	10 mg	

Example 24

Liposomes are prepared according to examples 4 - 14. The liposome dispersion (100 µl) is diluted to 5 ml with an 0.9 % NaCl solution and centrifuged at 25000 g for 15 min. The pellet is suspended in 1.5 ml of an aqueous solution of lactose (100 mg/ml) The dispersion is flash-frozen by dripping it into liquid nitrogen and is then lyophilized. The dry powder is dispersed in distilled water and the encapsulation of terbutaline sulphate into the liposomes is calculated according to the method described above.

		Encapsulation efficacy (%)
DPPC	10 mg	
Cholesterol	10 mg	26
Terbutaline sulphate	10 mg	

Example 25

11 g Epikuron 200H and 11 g terbutaline sulphate were dissolved in a mixture of 154 g t-butanol and 66 g distilled water under gentle heating. The solution was flash-frozen by dripping it into liquid nitrogen and was then lyophilized. 2.5 g of the resulting powder was dispersed in 197.5 g of an aqueous solution of lactose (3.8 weight %). Liposomes were formed by heating (maximum temperature 60°C) the sample for approximately 30 minutes during stirring. The liposome dispersion was spray-dried with a Buchi 190 Mini Spray-Dryer using an inlet temperature of 159°C. When an aqueous solution was added to the spray-dried powder, a liposome dispersion was reformed.

Release of β_2-receptor active substance from liposomes

Substantially all the non-encapsulated drug is removed from the continuous aqueous phase by centrifugation at 25000 g for 15 minutes and redispersion of the pellet in saline (0.9 % NaCl solution). The liposome dispersion (4 ml) is placed in a dialysis bag (Spectrapor Membran Tubing). The rate of release of β_2-receptor active substance from the

liposomes is determined by measuring the amount of drug in the liposomes after dialysis at 37 °C against 100 ml of saline. After various times the dialysis is stopped and the amount of β_2-receptor active substance in the dialysis bag is measured according to the method described above.

The results of this study are shown in Table 1.

Table 1 DIALYSIS OF FREE AND LIPOSOME ENCAPSULATED B_2-RECEPTOR ACTIVE SUBSTANCES.

Test preparation	% in dialysis bag at various times			
	1-1.5 h	3-4 h	6-7 h	16-22 h
TERB	52	17	n.d.	4
TERB-LIP	n.d.	82	79	72
MAB-LIP	94	74	62	43

TERB: Free terbutaline sulphate

TERB-LIP: Liposome encapsulated (DPPC, DPPA, cholesterol, 10:1:10 w/w) terbutaline sulphate

MAB-LIP: Liposome encapsulated (DPPC, DPPA, cholesterol, 10:1:10 w/w) mabuterol

n.d.: not determined

Already after 3 hours an equilibrium between the aqueous phase inside the dialysis bag and the external aqueous phase is established when terbutaline sulphate is dialysed. On the other hand, liposome-encapsulated mabuterol and terbutaline sulphate do not reach any equilibrium even after 20 hours of dialysis. These results show that it is possible to obtain a local retention of the active substance by encapsulation into liposomes.

Biological Tests

A Preparation of formulations for administration

DPPC (40 mg), DPPA (4 mg) and cholesterol (40 mg) are mixed in a glass tube. The components are dissolved in chloroform. The solvent is evaporated by the use of N_2 resulting in a thin film of the lipid components on the inner surface of the glass tube. Distilled water (4 ml) is added to the lipids. Formation of the liposomes is performed by sonication at a temperature above the phase transition temperature.

40 mg of terbutaline sulphate (or an other β_2-receptor active substance) dissolved in 2 ml distilled water is added to the liposomal dispersion and the mixture is frozen and freeze-dried.

The freeze-dried powder is hydrated in 400 µl distilled water at 60 °C for 30 minutes and diluted to appropriate concentration with saline. Approximately 40 % of the drug was encapsulated into the liposomes. This formulation was used for determination of anti-edema activity of Sephadex treated rats.

The liposome dispersion was centrifuged at 25000 g for 15 minutes in order to obtain a formulation where almost 100 % of the drug is encapsulated into the liposomes. This formulation was used for determination of protective effect against a histamine-elicited bronchospasm in guinea-pigs.

B Anti-inflammatory effect

Intratracheal instillation of Sephadex beads into rats leads to bronchial and also to alveolar inflammation (Källström, L. et al. Agents and Actions 1985 vol 17, 3/4, 355). This provokes interstitial lung edema, which in-

creases the lung weight, and the inflammation can be graded as the increase of the lung weight compared to a saline-instilled control group. The lung edema formation can be counteracted by pretreatment with β_2-receptor active substances, preferably by local adminstration as intratracheal instillation or by inhalation. Ideally an anti-inflammatory action should be obtained only at the site of drug application in the lung, but not in the rest of the body.

The differentiation between drug actions in the treated lung region and outside this area can be tested in the following way. Sprague Dawley rats (240 g) were slightly anaesthetized with ether and the β_2-receptor active preparation (in liposomes suspended in saline) in a volume of 0.5 ml/kg was instilled into just the left lung lobe. Two hours later a suspension of Sephadex (5 mg/kg in a volume of 1 ml/kg) was instilled in the trachea well above the bifurcation so that the suspension reached both the left and right lung lobes. 2 hours after Sephadex instillation the test preparation in a volume of 0.5 ml/kg was instilled into the left lung lobe. 16 to 20 hours later the rats were killed and the left and right lung lobes were dissected out and weighed separately. Control groups got saline instead of the test preparations and saline instead of Sephadex suspension to determine the weight of non-drug treated Sephadex edema and the normal lung weight.

As stated above an ideal β_2-receptor active substances should have a high pharmacological activity at the site of application in lung, but a low activity outside this area. Therefore, in the selected model an optimal preparation should have a high anti-edema activity in the locally pretreated left lung lobe and less activity in the right lung half.

The results of a comparative study is given in Table 2. The pharmacological profile of a liposomal formulation of terbutaline sulphate is compared that of free terbutaline sulphate.

Table 2 EFFECT OF FREE AND LIPOSOME ENCAPSULATED TERBUTALINE SULPHATE ON SEPHADEX INDUCED LUNG EDEMA IN RAT (N=6)

Preparation and dose		% Inhibition of lung edema	
	mg/kg	in treated left lobe	in right lobe
TERB	0.1	20	23
	1	43	41*
	10	95**	81**
TERB-LIP	0.01	36	5
	0.1	79*	47
	1	98**	65**

TERB: Free terbutaline sulphate

TERB-LIP: Liposome encapsulated (DPPC, DPPA, cholesterol, 10:1:10 w/w) terbutaline sulphate

*, ** = $P < 0.05$, 0.01, respectively, in comparison with control group

The liposomal formulation of terbutaline sulphate had a more selective activity for the application site in the lung than free terbutaline sulphate. The two test formulations more or less completely blocked the edema of the left lung lobe but the liposomal formulation was surprisingly coupled to only a moderate protective activity in the other lung lobe wheras free terbutaline sulphate completely blocked the edema of the right lung as well.

In addition to the observed separation between the anti-edema activity in the left and right lung lobe, terbutaline sulphate encapsulated into liposomes also shows a surprisingly high absolute potency for the action of the left lung lobe (~100 times more potent than free terbutaline sulphate).

Additional tests in this model have shown that procaterol, mabuterol and salbutamol encapsulated into liposomes show the same anti-edema profile as terbutaline sulphate encapsulated into liposomes, i.e. a 100-fold potentiation of the anti-edema activity at the site of application compared with free terbutaline sulphate.

C Bronchospasmolytic effect

Inhalation of aerosolized histamine to concious guinea pigs produces a dyspnea. The concentration of histamine to be aerosolized can be selected to produce a defined dyspnotic breathing within 2 min of exposure to histamine. Animals pretreated with inhaled bronchospasmolytic drug can be protected from the dyspnotic breathing (animals which withstand the dyspnea for more than 2 min). By administering β_2-receptor active substances at different time intervals before the histamine provocation and by measuring the protective effect it is possible to determine the duration of the activity of the substance.

Guinea pigs were exposed for 15 min to aerosolized terbutaline sulphate or to aerosolized liposome encapsulated terbutaline sulphate generated from a MA2 nebulizer with a terbutaline sulphate concentration of 1×10^{-3} M of the two formulations. The animals were exposed to the bronchospasmolytic agent 1, 2, 5 and 10 hours before the histamine challenge. The results of this study are given in Table 3.

Table 3 EFFECT OF FREE AND LIPOSOME ENCAPSULATED TERBUTALINE SULPHATE ON HISTAMINE INDUCED DYSPNEA IN GUINEA PIG.

Test preparation	Protective effect (sec) at various times 1h	2h	5h	10h
TERB	306	195	154	108
TERB-LIP	185	200	374*	150

TERB: Free terbutaline sulphate

TERB-LIP: Liposome encapsulated (DPPC, DPPA, cholesterol, 10:1:10 w/w) terbutaline sulphate

* = $P < 0.05$ in comparison with free terbutaline sulphate

Free terbutaline sulphate shows rapid onset of the a protective effect against histamine. A corresponding concentration of liposome-encapsulated terbutaline sulphate appears to have a delay in developing the same protective effect as the free terbutaline sulphate. When administered 2 hours before challenge the two used formulations of terbutaline sulphate have the same effect. However, there is only a limited protective effect of terbutaline sulphate when adminstered 5 hours before histamine provocation whereas liposome-encapsulated terbutaline sulphate surprisingly shows a maximal protection when the formulation is adminstered at this time. It can be concluded from this study that encapsulation of terbutaline sulphate into liposomes gives a prolonged duration of the bronchospasmolytic activity compared with equal amount of the free drug.

CLAIMS

1. A pharmaceutical composition characterized in that the preparation consists of a dry powder comprising liposomes and β_2-receptor active substance.

2. Pharmaceutical compositions as claimed in claim 1, designed for administration to the respiratory tract.

3. Pharmaceutical composition as claimed in any of claims 1-2, wherein the β_2-receptor active substance is entrapped within the liposomes.

4. A pharmaceutical composition as claimed in any of claims 1-2, wherein the β_2-receptor active substance is portioned between the liposomes and an external phase.

5. A pharmaceutical composition as claimed in any of claims 1 to 4 wherein the β_2-receptor active substance is selected from the following substances; terbutaline, salbutamol, mabuterol, fenoterol, orciprenaline, isoprenaline, formoterol, isoetharine, clenbuterol, hexoprenaline, procaterol, 1-(4-hydroxyphenyl)-2-[1,1-dimethyl-3--(2-methoxyphenyl)-propylamino]-ethanol, 1-(3,5-dihydroxyphenyl)-2-[1,1-dimethyl-3-(2-methoxyphenyl)propylamino]-ethanol, 1-(3,4-dihydroxyphenyl)-2-[1,1-dimethyl-3-(2-methoxyphenyl) propylamino]--ethanol, 4-hydroxy-α'-[[[6-(4-phenylbutoxy)-hexyl]-amino]-methyl]-1,3 benzyldimethanol or a pharmacologically acceptable salt thereof and mixtures thereof.

6. A pharmaceutical composition as claimed in claim 5 wherein the β_2-receptor active substance is terbutaline sulphate.

7. A pharmaceutical composition as claimed in any of claims 1 to 6 wherein the main liposome-forming lipid component is one or more phospholipids, optionally together with one or more other lipid components.

8. A pharmaceutical composition as claimed in claim 7 wherein the main liposome-forming lipid component is phosphatidylcholine, preferably selected from the following substances; dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine and mixtures thereof.

9. A pharmaceutical composition as claimed in any of claims 1 to 8 wherein the liposomes contain a sterol as stabilizer, preferably cholesterol or carbohydrate derivatives thereof in a proportion of 0.1 to 50 % w/w of the total lipids.

10. A pharmaceutical composition as claimed in any of claims 1 to 9 wherein the liposomes contain a substance which donates a positive or a negative charge, preferably selected from the following substances; phosphatic acid, dicetyl phosphoric acid, phosphatidyl serine, phosphatidyl inositol, phosphatidyl glycerol, phosphatidyl ethanolamine, stearylamine, stearyl amine acetate, cetylpyridinium chloride and mixtures thereof used in a proportion of 0.01 to 30 % w/w of the total lipids.

11. A pharmaceutical composition as claimed in any of claims 1 to 10 wherein the ratio by weight of β_2-receptor active substance to lipid is from 0.01 to 100, preferably from 0.1 to 10.

12. A pharmaceutical composition as claimed in any of claims 1 to 11 wherein the dry product is obtained by dehydration, preferably lyophilization or spraydrying.

13. A pharmaceutical composition as claimed in claim 12 wherein the dehydration is performed in the presence of a hydrophilic filler.

14. A method for the treatment and control of allergic, broncho-constricting, and inflammatory conditions in the respiratory tract in mammals, including man, characterized by the administration to the host in need of such treatment an effective amount of a composition as claimed in any of claims 1 to 13.

15. A process for the preparation of a dry powder according to claim 1 characterized by

i) mixing a liposome dispersion with a cryo-protective agent,

ii) rapidly freezing in liquid nitrogen, and

iii) dehydration.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE87/00148

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

A 61 K 9/14, 9/12, 9/72 4

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	A 61 K 9/00, /08, /10, /12, /14, /72, 47/00
US Cl	424:14, 19, 38, 199; 514:78

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	WO, A, 86/01714 (RIKER LABORATORIES INC) 27 March 1986 See claims 1, 15, examples 2, 4, page 4, lines 15-18, page 5, lines 27-31, page 7, line 7, page 12, lines 24-25. & EP, 0195809 JP, 62500643	1-3, 5-8, 11
Y	FR, A, 2 298 318 (TANABE SEIYAKU CO) 20 August 1976 See claims 1-2, example 1, page 4, lines 21-25. & DE, 2601207 US, 4016100 GB, 1487989 JP, 51086117	1, 4, 7-8, 11
Y	SE, A, 8404118-5 (STERWIN AG) 18 February 1985 See claims 1, 4, 6, example 31, page 6, lines 18-21, page 7, line 11, page 9, second paragraph. .../...	1, 2, 5, 6, 8-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1987-05-05	1987 -05- 18
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Agneta Tannerfeldt

L.E

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 14, because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by therapy (Rule 39.1.iv)

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- [ ] The additional search fees were accompanied by applicant's protest.
- [ ] No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A, 152 379 (LIBA-GEIGY AG) 21 August 1985 See claims 1, 2, 4, page 13, formula 1.5 and line 28, page 14, lines 20-22, page 30, lines 17-20. & JP, 60190710	1, 5
Y	EP, A, 170 642 (AB DRACO) 5 February 1986 claims 1, 4, examples 1, 2 page 2, lines 25-27. & JP, 61043110	1, 2, 8, 9, 12, 15
Y	GB, A, 1 575 343 (IMPERIAL CHEMICAL INDUSTRIES LIMITED) 17 September 1980 & NL, 7805005 BE, 866697 FR, 2390159 DE, 2818655 JP, 53142514 US, 4311712 AU, 514644 CA, 1114758 SE, 8201350 SE, 8201351 US, 4370349 SE, 440725 CH, 650944 CH, 652615	1, 7-10
Y	SE, B, 432 053 (BATTELLE MERORIAL INSTITUTE) 19 March 1984 See claims 1-4. & BE, 869551 GB, 2002319 NL, 7808204 FR, 2399241 DE, 2834308 LU, 80079 JP, 54049317 US, 4229360 CH, 621479 CA, 1114714 AU, 520915	1